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Morphological studies of the composition of the Green Turtle (*Chelonia mydas*) hyoid bones found in Peruibe, Southern Coast of Brazil, Mosaic of Conservation Units-Jureia-Itatins

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Abstract— *Chelonia mydas, popularly known as the green turtle, is widely distributed in tropical seas and uses as its feeding area the coastal region of Peruíbe, located on the southern coast of São Paulo State. It has a solid cranial formation, without temporal openings and formed by the junction of several small bones, among them the hyoid bones that have the function of supporting the tongue. In a study conducted with 10 individuals of the same species and with similar sizes, the objective was to verify the presence of a differentiated bone structure that has not been described in any study until the present moment and to verify if it was not only anatomical variation generated by any anomaly. in the evaluated animals. The techniques used for verification were computed tomography (with window for bone parts: center - WL 300 and width - WW 1,500) and histological analysis. For histology, the hyoid bone structure was removed from the skull separation and disarticulation and tongue extraction. With the separation of the bones, cuts of approximately 0.5 cm in length were made and fixed in 10% paraformaldehyde, with subsequent application of the fixation procedure, for observation with scanning electron microscopy. The structure analyzed was identified in 6 individuals. This proves that it is part of the formation of the hyoid bone, so it is necessary to name this structure and carry out in-depth studies to know its importance and to verify if it is a modification that is affecting the species or if it is a genetic variation that affected population studied.*

Keywords— keratobranchial process II; morphology; scanning electron microscopy; tomography.

I. INTRODUCTION

The *Chelonia mydas* (1) popularly known as the green turtle has wide distribution in tropical seas, usually between 40ºS and 40ºN (2). It uses as food area the coastal region of Peruíbe, located on the south coast of São Paulo that is within the areas of the Cananéia-Iguape-Peruíbe Conservation Units, Jureia-Itatins Conservation Units Mosaic and Tupiniquins Ecological Station. (3).

According to the latest IUCN (Red List of Threatened Species) assessment in 2004, the conservation status of the species Chelonia mydas (Family Cheloniidae) has category A2bd, which classifies the species as endangered, based on information on population reduction (4). With the increasing decline in the number of individuals, studies are needed to assist in the health of these animals when found / caught, whether intentionally, incidentally, or stranded both alive and dead. These individuals may be affected by various diseases involving the cranial region, such as meningeal hemorrhage, spinal cord compression, traumatic injuries associated with fishing activities, ingestion of hooks and lines, eye fibropapillomas, among others (5)(6).

Unlike other reptiles, the sea turtle has a solid skull, without temporal openings and formed by joining several small bones. It is made up of the neurocranium that provides the lining shell for the brain and the esplanocranium which is made up of the bones that cover the face. Anatomical

variation that promotes bone shape is characteristic for each species (7).

The green turtle has a cranial shape consisting of a short rounded muzzle lined with keratin and parietal bones with shallow carvings. They have a sturdy jaw, the upper one having a smooth U-shaped contour and the lower jaw having a ridge parallel to the interior surface and a midline cusp. The palate is present between the upper jaw margins and the inner nostrils (alveolar surface), has a pair of mountain ranges that occur parallel to the outer edge of the mandible. In addition, it has a horned or ranch-shaped beak that covers the maxillary, premaxillary, and vomer bone of the upper jaw, and the lower jaw dental, and present the hyoid bone, which has the function of supporting the tongue (8).

Computed tomography (CT) is a noninvasive imaging diagnostic method that has been the focus of research to develop new study and treatment methods to assist in the conservation of sea turtle species, as can be cited for use in rehabilitation Due to its high sensitivity, it allows the visualization of various organs, providing information on its shape, as well as the characterization of small alterations. In addition, it is possible to provide information on bone morphology, consequently allowing the acquisition of biomechanical parameters, as it characterizes loss of bone mass (9)(10).

It is widely used to detect skeletal and soft tissue disorders in turtles (11). This is because turtles exhibit changes in bone mineral metabolism regardless of whether they are free-living or captive animals (12)(13)(14).

CT has been used to study the embryonic development of these animals, according to Kuratani (15) who describes the development of the chondrocranium of *Caretta caretta* species. In the early stages of embryonic development, the morphology of bird and reptile embryos resembles that of a shark embryo, as does the initial pattern of mesenchymal condensation seen in mammalian skulls. Thus, reptiles can serve as a useful model system for understanding the development and evolution of the amniotic skull. They exhibit a set of primitive features that are characteristic of amniotes such as an extensively chondred neurocranium composed of paracordal cartilage and broad orbital cartilage that surrounds the neural tube (16).

In the application of the CT technique in the chondrocranium region of juvenile turtle juveniles, the presence of a bone structure that was not previously described was observed, and there are no studies related to the observation of sequential morphological changes in the hyoid bones of these animals. regardless of their stage of growth. Therefore, it will be necessary to study the morphology of the Chelonian hyoid to discover the function of this new structure found only in this species (*Chelonia mydas*).

The aim of the present study was to describe and name a new bone structure (keratobranchial process II), located in the 2nd branchial horn of the hyoid.

II. MATERIAL AND METHODS

We used 10 individuals of the species Chelonia mydas, found dead within the Environmental Protection Area - Cananeia-Iguape-Peruíbe and Cananéia-Iguape-Peruíbe Conservation Units, Mosaic of Jureia-Itatins Conservation Units, collected by the SOS Tartarugas Project, with license. Tamar / Icmbio-50132, authorization of the Ethics Committee No. 003/19 - CEUA IBIMM and donated to FMVZ, and approved by CEUA FMVZ – USP No. 3829270117 of 03/15/2017.

The animals were taken to the radiology and tomography center of the University Hospital of the University of São Paulo, where they diagnosed the images using the Philips Brilliance CT Scanner, 64 rows of detectors. Cutting Thickness: 1mm. Windows were obtained for soft tissue and bone tissue. Soft Part Windows: Center (WL 60) and Width (WW 400), Bone Part Windows: Center (WL 300) and Width (WW 1,500), Flat Reconstructed Cuts: Axial, Coronal, and Sagittal. The Volumetric Reconstruction Technique was designed with Volume Rendering and the computer programs used for visualization and capture were done through Philips Workstations and Radiant DICOM Viewer. For the hyoid bones only windows for bone parts were used (HU-University Hospital-Center of Radiology-USP).

The procedures for macroscopic analysis include the separation and disarticulation of the skull, with cuts made with the aid of a scalpel, sectioning the skin and musculature, for access to the tongue. After removing the tongue from the samples, the musculature and cartilage of the tongue were separated until the hyoid bone structure was located (17).

The samples were cut into approximately 0.5 cm long sections and fixed in 10% paraformaldehyde, dried in a Balzers CPD 020 critical point apparatus (CADI-FMVZ-USP), glued with carbon glue on aluminum metal bases (stub). and sputting with gold on the EMITECH K550 (FMVZ-USP) metallizer, and subsequently analyzed and photographed on a LEO 435VP scanning electron microscope (FMVZ-USP) (17).

III. RESULTS AND DISCUSSION

The animals had an average body mass of 5.4 kg and values of carapace curvilinear length (CCC) 38.05cm and carapace curvilinear width (CCL) 34.95cm, the individual measurements are shown in table 1.

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Table 1 - Biometric data of green turtles.

INDIVIDUALS	CCC (cm)	LCC (cm)	BODY MASS (Kg)
01	40	35	6,5
02	36	32	4,5
03	26	25	1,6
04	38	36	5,5
05	44,5	39,5	7,8
06	33	31	3,9
07	37,5	33	4,5
08	44	41	5,2
09	41,5	39	7,6
10	40	38	6,9

Source: WYNEKEN, (2001) p. 28

Anatomy is of great importance in wild veterinary medicine, most of the findings were based on the techniques of dissection and study of the bones of various animals, which contributes a lot to the morphological sciences. In the case of sea turtles, mainly *Chelonia mydas* species, there is no complete anatomical atlas, being used only as reference the works restricted to the production of complementary scientific articles. In this work, the anatomy and dissection techniques contributed to the discovery of a new structure, besides using computed tomography as an important research aid tool.

During the anatomical evaluation process of the skull bones of a green turtle (*Chelonia mydas*) individual, the presence of a new anatomical structure was observed and according to a bibliographic survey, it has not been cited in any world literature until the present moment, which generates the need to discuss a new nomenclature for it.

To verify its presence, the study was conducted with other animals, totaling ten individuals, through the computed tomography procedure in the skull region (Figure 1) and to verify if it was not only anatomical variation generated by any anomaly in the evaluated animal, 9 more individuals were dissected whose hyoid bones were evaluated. The animals evaluated were of the same species and with similar sizes.

Among all individuals analyzed, the structure was found in 6 individuals. Which proves that it is part of the formation of the hyoid bone. The bone composition of the hyoid apparatus follows in figure (2).

Fig.1 *- 3D computed tomography of the green turtle skull (Chelonia mydas). Figures I, II and III - yellow circle, presence of the new structure. Caption: In (A): I - anatomical section for removal of the hyoid bone, I - keratobranchial I, II - hyode body, III - keratobranchial II. in (b):, IV- horned beak, II - tongue, III - keratobranchial musculature. in (c): VII -lingua, VIII-hyoid paraparate (keratobranchial I or horn I), IX-keratobranchial II (horn II). in (d): X - body of the hyode, XI - keratobranchial I, XII - keratobranchial. in d, e, f (circle) keratobranchial process II.*

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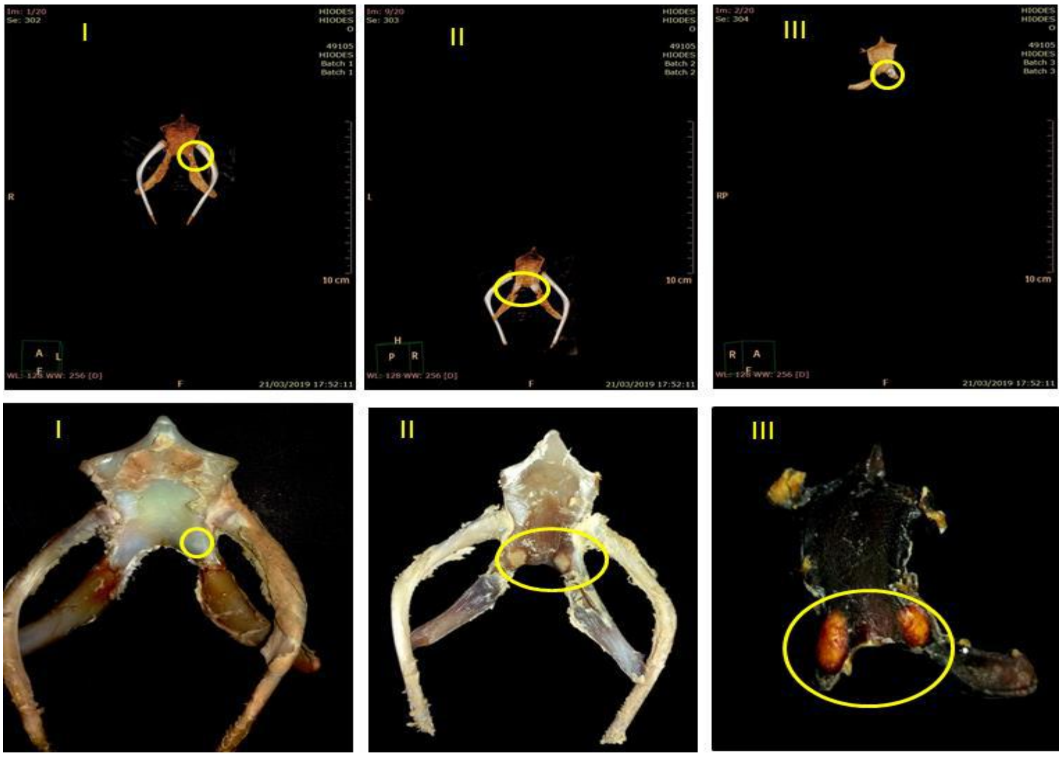

Fig.2: Computed tomography of the bone structure present in the green turtle hyoid. It is observed in the upper figure in I, II and III (yellow circle) the presence of white bone, orange cartilage. In the lower figure, photographs of the hyoid bone, in I, II and III (circles) presence of bone structures. Processed in a CT scanner at the Radiology Center of the University Hospital of the University of São Paulo.

In computed tomography it was possible to prove that the structure is a bone. Layers define a different composition of structures and coloring. It is possible to visualize the porous layers of the bones according to figures 3 and 4.

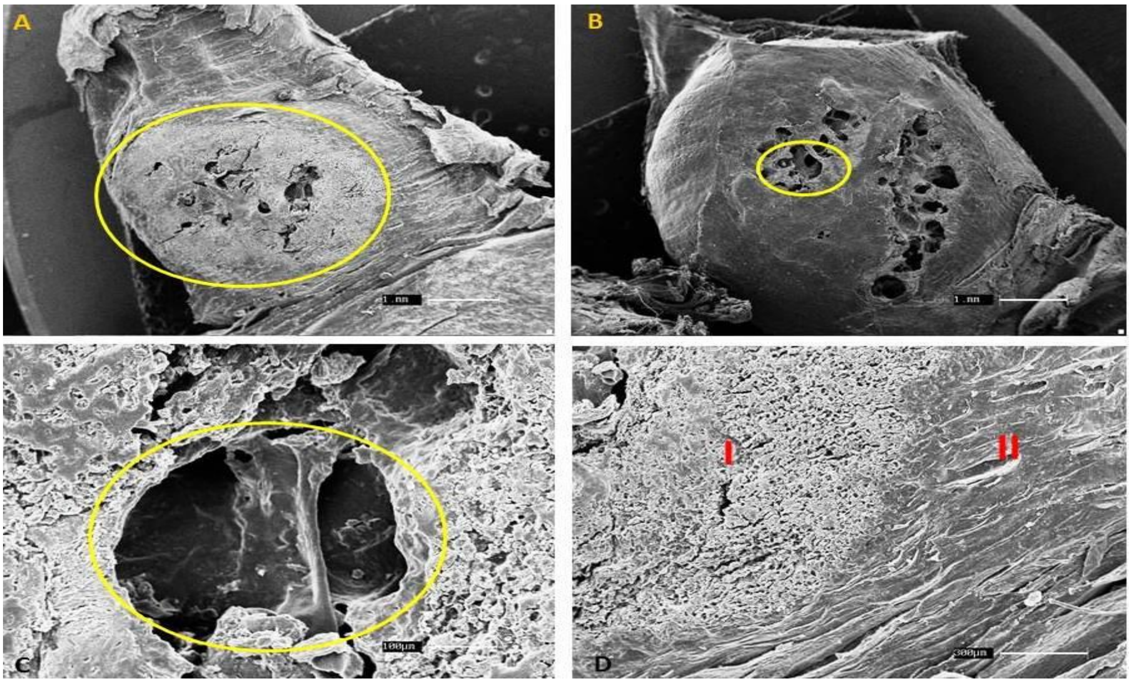

Fig.3: Computed tomography of the keratobranchial process II; In A (yellow circle) calcified bone, 1µm increase; in B - Circle (pores) 1µm increase; in (C) porous channels, 100µm increase; in (D) I - bone structure, in II - cartilage-increase of 300µm.

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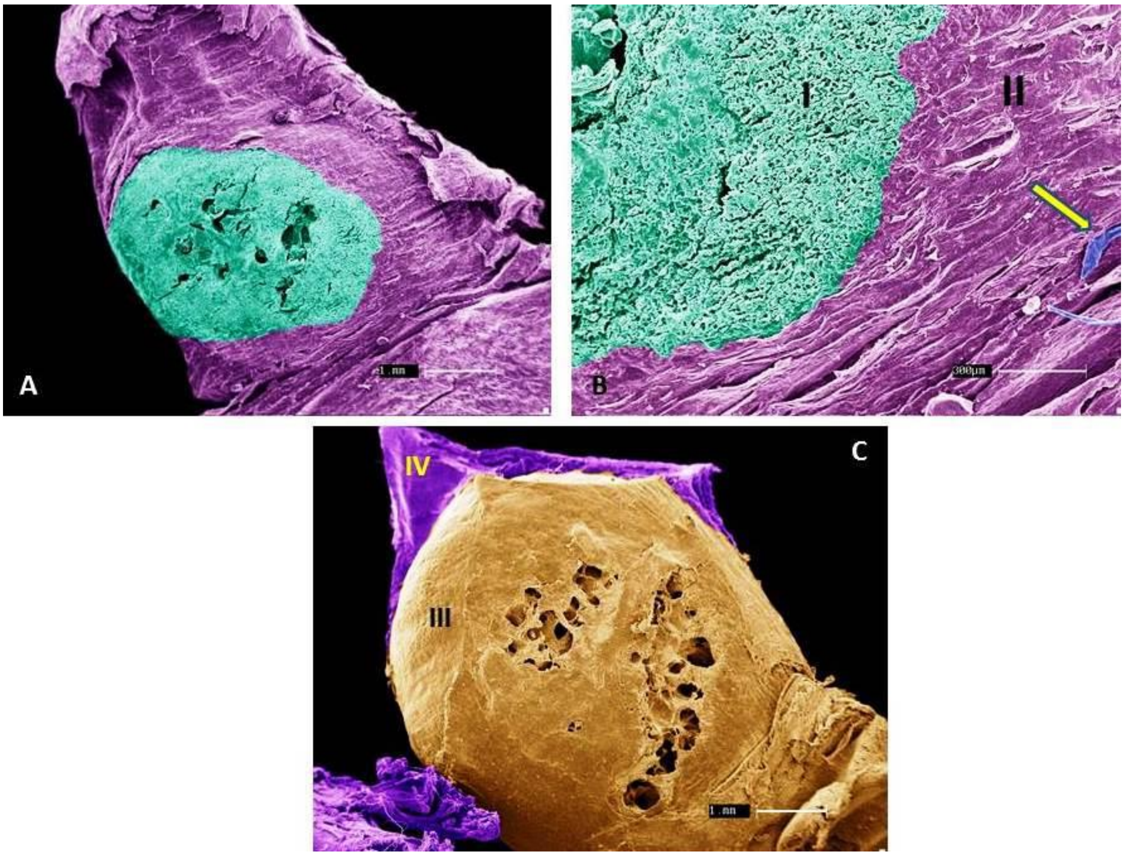

Fig.4: Scanning electron microscopy of the new color structure. Note (A) green (calcified bone and cartilage violet) 1µm increase; in (B), I - calcified bone, II - cartilage, arrow (muscles) increase of 300µm; and in (C) III - bone calcified with pores, IV - cartilage - 1µm increase.

In works by Kuratani (16)(18)(19) describes cranial morphology and development of the hyoid in the skull of Caretta caretta species, but did not present at any stage of developmental stages (from embryo to adult animal) the appearance of the observed structure. on green turtles.

Other authors such as Werneburg *et al.* (20), Garcia *et al.* (21), Jones *et al.* (22) describe the skull and hyoid bone of *Caretta caretta* and *Lepdichoelys kempi* turtles with computed tomography technique; Wyneken (8) described the anatomical part from dissection and Arencibia *et al.* (23) describe the skull of the species Caretta caretta from computed tomography and also did not observe the presence of the structure present in the hyoid bone apparatus, located in the second gill horn or keratobranchial bone II, of the green turtle.

Work (24) and Wyneken (8) cite in their work peculiar changes in green Hawaiian tortoises, such as the emergence of a new structure called (esophageal diverticulum or crop), the function of which has not yet been confirmed. This structure is found only in green turtles in the Caribbean regions and also in some individuals in South America.

IV. CONCLUSION

Veterinary anatomy and osteology are essential study tools to develop and expand the knowledge about marine animals that are necessary to understand the life dynamics of these organisms and therefore have data to use for their conservation. At the first moment it is very important to characterize this structure and name it "keratobranchial process II". Defining the importance / function of the bone discovered in the green turtle may help in understanding the eating habits of these animals, which is completely different from other species of sea turtles, so perhaps not yet described or found in them, as well as verifying if it is. a modification that is affecting the species or if it is a genetic variation that affected the population studied This opens a new door to study the function of this structure and why it does not appear in all green turtles.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

REFERENCES

[1] LINNAEUS CN, *Chelonia mydas*. 1758.
[2] HIRTH, H.F. Synopsis of the biological data on the green turtle, *Chelonia mydas* (Linnaeus 1758). Washington: United States Fish and Wildlife Service Biological Report, 120p. 1997.
[3] LOPES, E. Q.; MILK C. S.; SILVA, C. S. A.; MELO, L. F.; FANNELI, C. Food content analysis of green turtles (Chelonia mydas) killed in strandings on the Peruíbe Coast, south coast of São Paulo. In: International Seminar Plastic Free Oceans, 1, Santos, 7 to 8 jun. 2018. Proceedings of the 1st International Seminar Free Oceans of Plastics. Santos: Unisanta Bioscience. P. 77-98, 2018.
[4] IUCN, 2019. The IUCN Red List of Threatened Specie. *www.iucnredlist.org*. Consultado em 20 de julho de 2019
[5] BROOKS, H.E., DOSWELL III, C.A., COOPER, J. On the environments of tornadic and nontornadic mesocyclones. Am. Meteorol. Soc. 9, 606–618. Brooks, H.E., Doswell III, C.A., Kay, M.P., 2003a. Climatological estimates of local daily tornado probability. Wea. Forecasting 18, 626–640. 1994
[6] ORÓS, J. *et al*. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). Diseases of aquatic organisms, v.63, p.13-24, 2005.
[7] ROMER, A.S. Osteology of the Reptiles. Krieger Publishing Group. Florida 772 pp. 1956.
[8] WYNEKEN, J. The Anatomy of Sea Turtles. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470, 1-172 pp. 2001.
[9] ALVES, L. C. Computed Tomography of the Thoracic Cavity of the Perro (Family Kennels L.) By Apparatus of Sixth Generation and Oral and Vascular Contrast Means - Murcia. Doctoral Thesis, Murcia Univ., Murcia, Spain. 2004.
[10] OLIVEIRA, J. F. D. *et al.* Densitometry of the dorsal vertebra, pleural bone and neural bone in healthy green turtles by quantitative computed tomography.Cienc. Rural, Santa Maria, v. 42, no. 8, p. 1440-1445, Aug. 2012 Available at (http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782012000800018&lng=en&nrm=iso). Viewed on 4/20/2019.
[11] GUMPENBERG, M.; HENNINGER, W. The use of computed tomography in avian and reptile medicine. Seminars in Avian and Exotic Pet Medicine, v.10, no.4, p.174-180, 2001.
[12] MADER, D. R. Reptile Medicine and Surgery. W.B. Saunders Company. Second Edition, Florida. 2006.
[13] GEORGE RH. Health Problems and Diseases of Sea Turtles. In: Lutz P and Musik JA (eds) The Biology of Sea Turtles, CRC Press, Boca Raton, Florida. Chap 14:363-385. 1996.
[14] ADAMS, J.E. Quantitative computed tomography. European Journal of Radiology, v.71, p.415-424, 2009. Available at http://www.sciencedirect.com/science/article/pii/S0720048X09004343. Viewed on 4/20/2019.
[15] KURATANI, S.; HORIGOME N.; AND HIRANO S. Developmental morphology of the cephalic mesoderm and re-evaluation of segmental theories of the vertebrate head: evidence from embryos of an agnathan vertebrate, Lampetra japonica. Dev Biol 210:381–400. 1999.
[16] KURATANI S. Development of the orbital region in the chondrocranium of Caretta caretta. Reconsideration of the vertebrate neurocranium configuration. Anat Anz 169: 335–349. 1989.
[17] MELO, LF; CABRERA, ML; RODRIGUES, ACB; TURQUETTI, AOM; LOPES, EQ; RICI, REG. Morphological Description of the Green Turtle Tongue (*Chelonia mydas*). International Journal of Advanced Engineering Research and Science (IJAERS) [Vol-6, Issue-5, May- 2019] https://dx.doi.org/10.22161/ijaers.6.5.39 ISSN: 2349-6495(P) | 2456-1908(O). 2019.
[18] KURATANI S, TANAKA S, ISHIKAWA Y, ZUKERAN C. Early development of the hypoglossal nerve in the chick embryo observed by the whole-mount nerve staining method. Am J Anat 182: 155-168. 1988.
[19] KURATANI S, MATSUO I, AIZAWA S. Developmental patterning and evolution of the mammalian viscerocranium: Genetic insights into comparative morphology. Dev Dyn 209: 139-155. 1997.
[20] WERNEBURG I. Temporal bone arrangements in turtles: No overview. J. Exp. Zool. (Mol. Dev. Evol.) 318: 235–249. 2012.
[21] Garcia KC, Gapin L, Adams J, Birnbaum M, Scott-Browne J, Kappler J, Marrack P. Immunity. doi: 10.1016/j.immuni.2012.05.018. Published online June 14, 2012.
[22] JONES MEH, WERNEBURG I, CURTIS N, PENROSE R, O'HIGGINS P, *et al.* The Head and Neck Anatomy of Sea Turtles (Cryptodira: Chelonioidea) and Skull Shape in Testudines. PLoS ONE 7 (11): e47852. 2012.
[23] ARENCIBIA, A., RIVERO, M.A., de MIGUEL, I., CONTRERAS, S., CABRERO, A., & ORÓS, J. Computed tomographic anatomy of the head of the loggerhead sea turtle (*Caretta caretta*). Research in Veterinary Science, 81 (2), 165–169. 2006.
[24] WORK, T.M. Marine Tortuga Necropsy Handbook for Biologists in Refugees or Remote Areas. National Wildlife Health Center, Hawaii Field Station. 25pp. 2000.